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Echinosphaeria pteridis sp. nov. and its *Vermiculariopsiella* anamorph

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Abstract – *Echinosphaeria pteridis* sp. nov. and its anamorph, *Vermiculariopsiella pteridis* sp. nov., was isolated as an endophyte from a pteridophyte, *Pteris vittata*, collected from the Western Ghats in India. This is the second report of culture-based teleomorph-anamorph connection in *Vermiculariopsiella*.

Key words – biodiversity, fungi

Introduction

During studies of microfungi of the Western Ghats in southern India (Keshavaprasad et al. 2003, Gawas et al. 2006, Pratibha & Bhat 2008), an interesting endophyte was isolated from the fresh rachis of a pteridophyte, *Pteris vittata* L. (family: *Pteridaceae*), collected from Gersoppa, Uttara Kannada District, Karnataka State. After isolation into pure culture, the fungus first produced a sporodochial anamorph within 7 days. A further 15 days of incubation at 23–25ºC led to the development of perithecia in small groups on tiny stromata amongst the sporodochia. The anamorph was identified as a new species of *Vermiculariopsiella* Bender (Bender 1932). The teleomorph was similar to *Echinosphaeria* A.N. Mill. & Huhndorf (Miller & Huhndorf 2004), but differed from the two known species (Gawas et al. 2006).

Materials and methods

Fresh and apparently disease-free rachises of *Pteris vittata* were processed for endophytes as described by Sati & Belwal (2005). The rachises were surface sterilized in 0.01% sodium hypochlorite (3–6 min) followed by 96% ethanol (30 s). The surface sterilized rachises were thoroughly rinsed thrice in sterile distilled water to remove all traces of surface sterilizing agents, cut into 0.1–0.5 cm pieces using a sharp sterile blade and plated on 2% malt extract agar (MEA, Himedia) plates with a mixture of antibiotics (bacitracin 20 mg, neomycin 20

mg, penicillin 20 mg, streptomycin 20 mg and tetramycin 40 mg dissolved in 10 mL of sterile distilled water added to 1 L of MEA medium). The mycelia emerging from the cut ends of the rachises were transferred onto fresh MEA plates (Borosil, 9 cm diam.). The plates were incubated at 23–25°C in diurnal light for several weeks until sporulation occurred.

Standard Deviation was calculated using statistical tools of Microsoft Excel.

Confirmation of anamorph-teleomorph connection

A sporodochium and perithecium, which developed in culture, were aseptically transferred onto separate flame sterilized slides and dissected in a drop of sterile distilled water to prepare spore suspension. The suspension was spread onto the surface of 2% malt extract agar plates. Germinated spores were aseptically transferred onto fresh MEA plates, incubated at 25°C until growth and sporulation occurred. The development of anamorph and teleomorph in culture arising from both conidia and ascospores were in conformity with *Vermiculariopsiella pteridis* and *Echinosphaeria pteridis*.

Taxonomy

Echinosphaeria pteridis S. Dharg. & Bhat, **sp. nov.** (Fig. 1–3, 7)
MycoBank MB 512784

anamorph — ***Vermiculariopsiella pteridis*** S. Dharg. & Bhat, **sp. nov.** (Fig. 4–6, 8)
MycoBank MB 512869

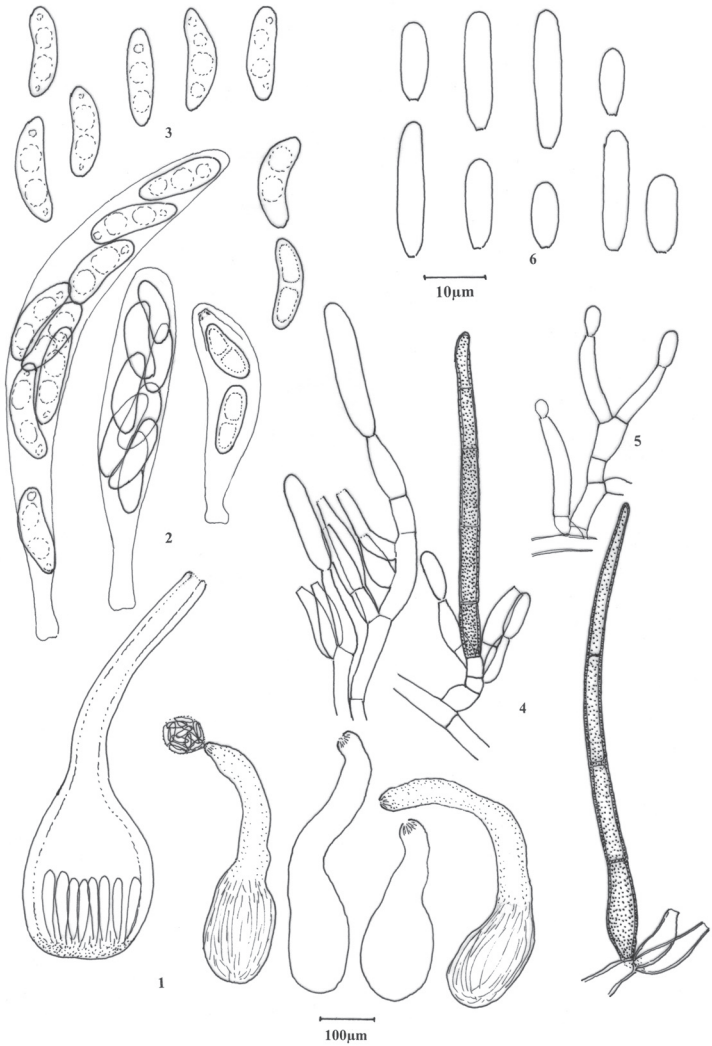
In MEA-cultura, coloniae 6–8 cm diametro in 10 dies. Ascocarpis 350–620 × 65–130 µm, collum 145–430 µm longus, 50–60 µm latum, 100 µm latum ad basim, pallide brunnea, glabratus. Asci octospori, 23–70 × 8–15 µm. Ascosporae aseptatae, laevia, hyalinae vel pallide brunneae, 10–23 × 4–8.5 µm, allantoideae vel ovalis, teres ad duo extremitas, interdum cum duo extremitas apiculata.

Anamorphosis: Conidiomata sporodochia, 580–1115 × 400–1015 µm, conidis agglutinates, cremea vel persicinus. Setae atro-brunnea, 40–145 µm longae, 1.5–3.5 µm latis ad medius, 2–6.5 µm latis ad pessum. Conidiophora parce ramosa, hyalina vel pallide brunae, 8–74 × 3–4.5 µm. Cellulae conidiogenae monophialideae, hyalinae, cylindricae vel lageniformes, 7–40 × 3–4.5 µm, sine emineo collarettae. Conidia aseptata, hyalina, oblongus vel cylindrica, 10–27 × 4–5.5 µm.

Holotype: On rachis of *Pteris vittata* as endophyte, Gersoppa, Western Ghats, Karnataka, India, 03-XII-2007, S. Dhargalkar, dried culture mat GUBH SD-469, Herb. No. HCIO 48775.

Etymology: Refers to the pteridophyte host genus, *Pteris*

Colonies on MEA after 10 days effuse, smooth, flat, cottony, moderate to fast growing, attaining a diam. of 6–8 cm, at first producing slimy sporodochial conidiomata, later with ascomata developing in groups on stromatic bases; margin fringed, off-white later turning grayish; reverse off-white. Mycelium superficial, with branched, septate, smooth, hyaline, hyphae 2–2.5 µm wide.



Figs. 1–3. *Echinosphaeria pteridis.* Ascocarp, asci, ascospores. Figs. 4–6. *Vermiculariopsiella pteridis.* Sporodochia with setae, conidiophores, conidiogenous cells and conidia.

Teleomorph: Ascomata perithecial, aggregated in groups on stromatic bases, pyriform, ostiolate, dark brown, 350–620 × 65–130 µm, with a conspicuous long neck bearing apical ostiole; neck pale brown, glabrous, 145–430 µm long, 50–60 µm wide above, up to 100 µm wide at the base. Asci unitunicate, 8-

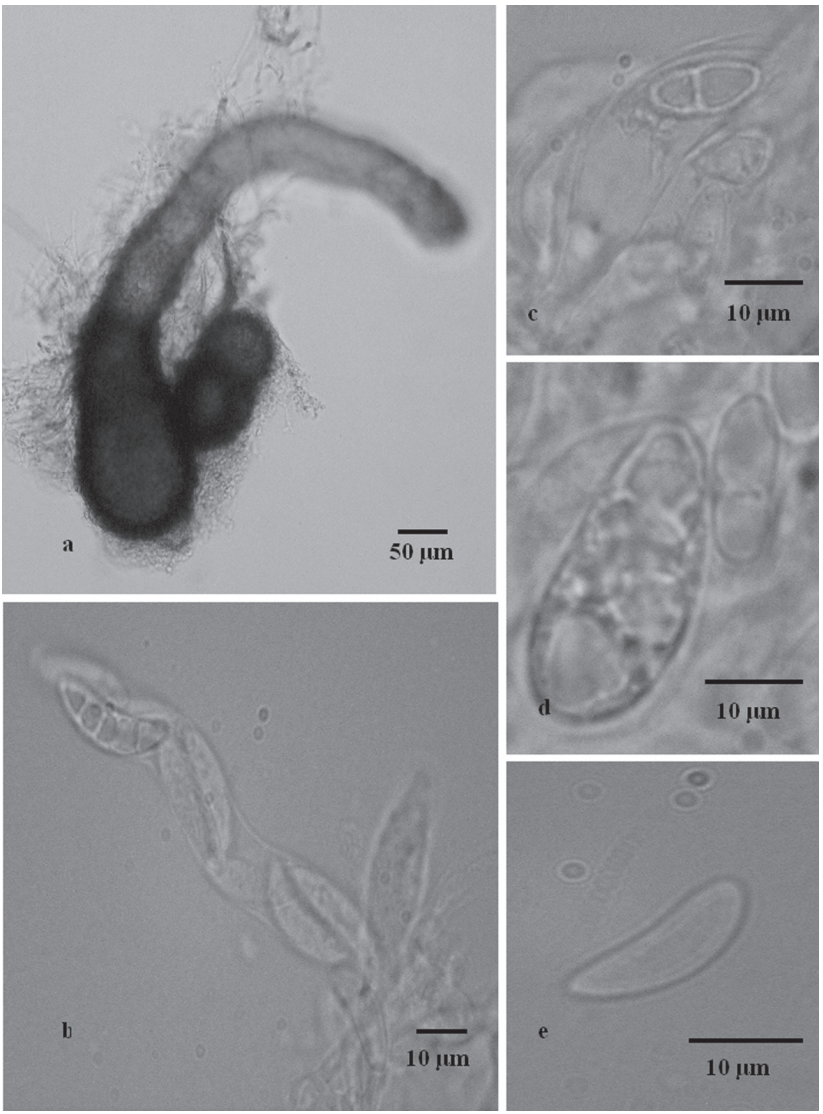


Fig. 7. *Echinosphaeria pteridis* a. ascocarp; b. ascus with biseriately arranged ascospores; c. immature ascus with conspicuous apical ring; d,e. ascospores.

spored, clavate, rarely ovoid, 23–70 × 8–15 µm, slightly narrower towards the tip, pedicellate, non-amyloid, with a conspicuous apical ring. Paraphyses absent. Ascospores aseptate, allantoid to oval, rounded at both ends, sometimes with slightly pointed ends, smooth, hyaline, turning pale brown with age, guttulate,

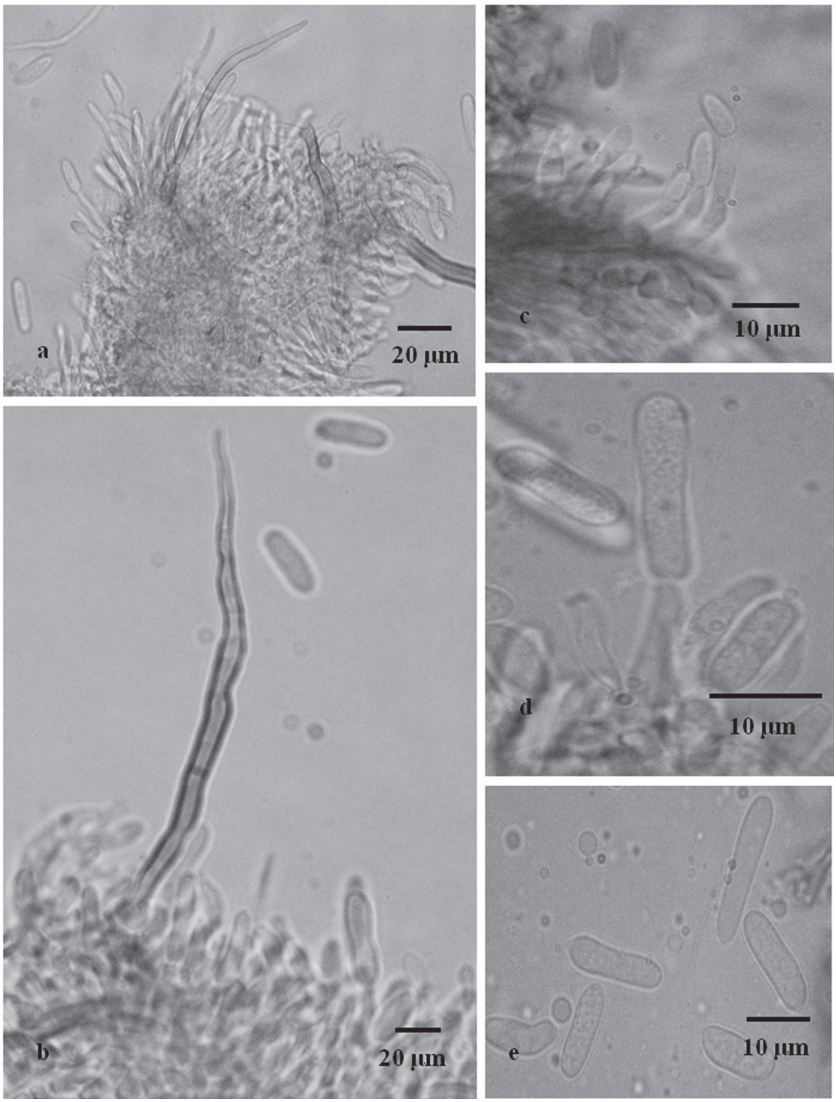


Fig. 8. *Vermiculariopsiella pteridis* a. sporodochial conidioma with setae, conidiophores and conidia; b. seta; c,d. conidiogenous cell with conidia; e. mature conidia.

10–23 (mean 17 ± 3) × 4–8.5 (mean 6 ± 1.5) µm, biseriately arranged in the asci.

Anamorph: Conidiomata sporodochial, developing on a pseudoparenchymatous stromatic base, scattered, 580–1115 × 400–1015 µm,

punctiform, globose, sometimes coalescing to form irregular patches, setose, with cream to peach-colored mass of conidia. Setae numerous, smooth, unbranched, 2–5-septate, dark-brown, erect, slightly curved to flexuous, rounded to pointed apex, arising from swollen basal cells, 40–145 µm long, 1.5–3.5 µm wide in the middle, 2–6.5 µm wide at the base. Conidiophores straight, slightly curved to flexuous, smooth, septate, sparsely branched, hyaline to pale brown, 8–74 × 3–4.5 µm. Conidiogenous cells monophialidic, determinate, integrated or discrete, hyaline, smooth, cylindrical to lageniform, 7–40 × 3–4.5 µm, without a conspicuous collarette. Conidia aseptate, solitary, smooth, hyaline, oblong to cylindrical, rounded at both ends, slightly narrower and truncate at base, 10–27 (mean 17.5 ± 4.5) × 4–5.5 (mean 4.5 ± 0.5) µm.

Discussion

The genus *Echinosphaeria,* typified by *E. canescens* (Pers.) A.N. Mill. & Huhndorf, is characterized by the production of subglobose to ovoid ascomata, with unitunicate asci containing allantoid, guttulate, biseriately arranged, smooth-walled ascospores (Miller & Huhndorf 2004). The genus includes species with three different genera of anamorphs, viz. *Endophragmiella* B. Sutton, *Selenosporella*-like synanamorphs (Hughes 1979, Sivanesan 1983, Miller & Huhndorf 2004) and *Vermiculariopsiella* (Gawas et al. 2006). *Echinosphaeria pteridis* differs from the two other species in the genus (Table 1) — namely *E. canescens* and *E. macrospora* Gawas, Bhat and K.D. Hyde (Gawas et al. 2006) — by its larger ascocarps, longer perithecial necks, and the sizes of asci and ascospores.

The hyphomycete genus *Vermiculariopsiella*, typified by *V. immersa* (Desm.) Bender (Bender 1932), has 12 reported species (Gawas et al., 2006). The genus is characterized by the production of superficial, setose sporodochia, with simple or branched conidiophores bearing monophialidic, subcylindrical to lageniform conidiogenous cells with collarettes, producing slimy masses of hyaline, aseptate conidia (Bender 1932). Species-delineation is based on differences in size and shape of setae, conidiophores, conidiogenous cells and conidia (Gawas et al. 2006). Four species are reported from India (Keshavaprasad et al. 2003, Gawas et al. 2006).

Among the species of *Vermiculariopsiella*, *V. pteridis* is somewhat similar to *V. elegans, V. parva, V. indica* and *V. endophytica* in having unbranched setae and cylindrical conidia with rounded ends. *V. pteridis*, however, differs from *V. elegans, V. indica* and *V. endophytica* in having smaller sized conidia and from *V. parva* in sizes of conidioma and conidiogenous cell. Although identical in spore size, the type species, *V. immersa*, differs from *V. pteridis* in having recurved conidiogenous cells with a cylindrical neck and flared collarettes.

Table 1. Comparison of *Echinosphaeria* species

Species	Ascocarp	Neck	Ascus	Ascospores	Reference
E. canescens	Subglobose to ovoid, dark brown, 500 µm diam	Inconspicuous	Cylindric-clavate, 10–12 µm diam	Uniseptate, 20–28 × 4.5 µm	Miller & Huhndorf (2004), Saccardo (1883)
E. pteridis	Pyriform, dark brown, 350–620 × 65–130 µm	Conspicuous, cylindrical; length: 145– 430 µm diameters: 100 µm (base), 50–60 µm (upper)	Clavate (rarely ovoid), 23–70 × 8–15 µm	Aseptate, 10–23 × 4–8.5 µm	Present study
E. macrospora	Pyriform, black, 410–490 × 150–265 µm	Inconspicuous	Clavate, 120–165 × 14–17.5 µm	Aseptate, 41–45 × 6–11 µm	Gawas et al. (2006)

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